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WATER AND METABOLIC RELATIONS OF CAVE-ADAPTED AND EPIGEAN LYCOSID SPIDERS IN HAWAII[1]

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ABSTRACT

Water loss rates, cuticular permeability, metabolic rates and rhythms were determined for the troglobitic spider, *Lycosa howarthi*, and an undescribed epigean spider, *Lycosa* sp., collected from lava tube caves and lava flows, respectively, on the Island of Hawaii. The cuticular lipid and hydrocarbon surface densities as well as an analysis of the hydrocarbon fraction were also determined for each species and correlated with their different cuticular permeabilities.

Water loss for the cave species was significantly higher at each test humidity, with the maximum difference between the species occuring at 19°C and 0% RH (11.14 ± 1.76 mg g^{-1} h^{-1} vs 1.01 ± 0.33 mg g^{-1} h^{-1}). Water loss rates decreased with decreasing saturation deficit between 0 and 70% RH in the cave species, but were relatively independent of RH in the epigean species. The mean metabolic rate for a 12-h period between 1500 and 0300 h was approximately one and one-half times greater in the epigean species (173.72 ± 6.22 μl 0_2 g^{-1} h^{-1} vs 115.71 ± .89 μl 0_2 g^{-1} h^{-1}). Oxygen consumption exhibited an increased trend in the epigean species during hours of darkness; 0_2 consumption rates for the cave species were very constant over the 12-h period.

The surface densities of cuticular lipid and cuticular hydrocarbon were significantly greater in the epigean species on both an individual spider and weight-specific basis. The hydrocarbon fraction of both species was comprised of numerous components, all saturated, ranging in chain length from 18-19 to over 41 carbon atoms. Straight-chain (*n*-alkane) molecules accounted for almost 65% of the total hydrocarbon fraction in the cave species, but only 23.5% of the total in the epigean species.

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INTRODUCTION

Most deep cave zones are characterized by perpetual darkness, stable temperatures, and constant high humidity. In view of these conditions, it has often been assumed that species restricted to these zones (troglobites) exhibit physiological responses that mirror the physical environment. Studies of cave insects and millipedes which show that they tend to select microenvironments that are high in relative humidity and relatively cool (Mitchell 1971, Bull and Mitchell 1972, Wilson 1975) add credence to this assumption. Experimental data, however, are still insufficient to permit firm conclusions regarding preference and tolerance capacities.

The unique cave faunal community discovered in lava tubes on the Hawaiian Islands (Howarth 1972, 1973) not only provides an opportunity to examine thermal, water and metabolic relations of troglobitic arthropods, but also permits comparison with closely related, perhaps ancestral, epigean species which exist near lava caves. One such example involves wolf species (Lycosidae). *Lycosa howarthi* Gertsch, the largest terrestrial troglobite in lava tubes on Hawaii Island, exhibits loss of pigment and has vestigal, probably non-functional eyes. A second, presently undescribed lycosid spider (*Lycosa* sp.) occurs on the surface of younger lava flows on Hawaii Island in some cases adjacent to the entrances of lava tubes inhabited by *L. howarthi*. This epigean species possesses the very large eyes typical of lycosids and is subjected to much greater fluctuations in temperature and humidity than its troglobitic congener.

This paper contains data on water loss rates, cuticular permeability, and metabolic rates and rhythms in these two spider species. Cuticular lipid and hydrocarbon surface densities as well as an analysis of the hydrocarbon fraction were determined for each species and correlated with observed differences in cuticular permeability.

METHODS

Spiders were collected on the Island of Hawaii between 1 September and 1 November 1979. Specimens of *Lycosa howarthi* came from the deep cave zone of Kazumura Cave (elev. 300–400 m) where temperatures remain between 19 and 20°C. The specimens were all mature or nearly mature females. The epigean lycosid was collected at night on the largely unvegetated lava flows on Kilauea Volcano between 800 and 950 m within Hawaii Volcanoes National Park. The specimens of *Lycosa* sp. were mostly mature or nearly mature females.

Cave spiders were maintained in the laboratory in individual plastic vials at 19°C and 100% RH; surface spiders were kept in individual vials at room temperatures (ca. 25°C) and humidities (ca. 50-60% RH). Water loss rates were determined gravimetrically on spiders placed inside a sealed glass desiccator maintained at 19°C and at relative humidities of approximately 0, 50, 70 and 90%, the latter generated by either Drierite (0%) or saturated salt solutions (Winston and Bates 1960). Relative humidity during each exposure was monitored by a standard laboratory hygrometer located inside the desiccator. A 5-hr incubation period was used in all tests.

Respiration was measured at 19°C using Gilson respirometer flasks containing 0.5 ml of 10% KOH to absorb CO_2. The flasks were lined with water-soaked tissue paper to maintain a near-saturated atmosphere during the exposures. A black plastic sheet was placed over the respirometer during all experimental runs to exclude light.

Table 1.–Lipid/hydrocarbon quantities extracted from the epicuticles of *Lycosa howarthi* and *Lycosa* sp.

Species	N	Pooled weight (g)	Total lipid (mg)	Hydro-carbon (HC) (mg)	Lipid/ spider (mg)	HC/ spider (mg)	HC/ lipid (%)
Lycosa sp. (epigean)	12	1.3096	2.12	0.24	0.18	0.02	11.3
L. howarthi (cave)	9	1.7575	1.11	0.13	0.12	0.01	11.7

Epicuticular lipids were removed by slurrying the spiders (pooled) in redistilled hexane for two 10-min perods. The lipid extract was filtered, evaporated to dryness under nitrogen, and weighed to 0.01 mg. Lipid classes present were determined by spotting a 1 μl sample of the extract on 0.25 mm Silica Gel G coated glass plates (TLC) and developing the plates in hexane:diethyl ether:formic acid (80:20:2, v/v). Lipid bands were detected by charring and identified against known standards. Hydrocarbons were separated from the other lipid classes by eluting the lipid extract with hexane through silicic acid columns (Jackson et al. 1974). The amount of hydrocarbon was determined gravimetrically after drying under nitrogen and an aliquant spotted on silver nitrate impregnated TLC plates to check for unsaturation. The hydrocarbon fraction was analyzed by gas chromatography (GLC) using 6′ by 1/8″ in. glass columns packed with 3% OV-101 on 100/120 Gas Chrom Q and programmed from 200 to 300°C at 4°C/min. Peaks were identified by comparison to retention times of standards and quantified by electronic integration. Branched components were identified on the basis of franctional equivalent chain lengths, as there was insufficient material to permit separation of *n*-alkanes and branched alkanes using the standard molecular sieve technique (Hadley and Jackson 1977).

RESULTS

Water Loss.–The water loss rates (WLR) for *Lycosa howarthi* (troglobitic) and *Lycosa* sp. (epigean) spiders are compared in Fig. 1. WLR were significantly higher ($P < 0.01$) for the cave species at each humidity, with the maximum difference occurring at the highest saturation deficit (0% RH). At 19°C and 90% RH, four of six epigean spiders exhibited a slight gain in body mass. The net result was an apparent water gain for this test group, whereas all cave spiders under identical conditions continued to lose water. WLR for the cave species were also significantly higher at each humidity when rates were expressed per unit surface area. Species differences in area-specific WLR followed the pattern observed for weight-specific determinations.

Oxygen Consumption.–Metabolic rates for the two spider species for a 12-h period between 1500 and 0300 hours are presented in Fig. 2. The mean weight (± 1 SD) for the troglobitic *L. howarthi* was 184.4 ± 16.0 mg (n = 4) versus 123.0 ± 24.0 mg for the epigean *Lycosa* sp. (n = 4). Oxygen comsumption expressed per unit spider mass was significantly higher ($P < 0.01$) for the epigean spiders at each hour of measurement. The mean value (± 1 SE) for the 12-h period for epigean spiders was 173.72± 6.22 μl 0_2 g^{-1} h^{-1} compared to 115.71± .89 μl 0_2 g^{-1} h^{-1} for the troglobitic spiders. These data indicate a metabolic rate that is approximately 1.5 times greater in the surface-dwelling spiders. In

the latter group there also was a trend for increased metabolism in hours which correspond to darkness in their natural habitat. In contrast, oxygen consumption rates were very constant for the cave species over the entire 12-h period.

Cuticular Lipids.—Chromatographic separation of extracted epicuticular lipids indicated the presence of hydrocarbons, wax esters, alcohol, sterols (principally cholesterol), triglycerides and free fatty acids in both spider species. Lipid-hydrocarbon quantities for the two species are given in Table 1. Epigean spiders (pooled) had 1.9 times more total lipid and 1.8 times more total hydrocarbon than the cave spiders. On a per individual basis, an epigean spider had 1.5 times as much lipid and twice as much hydrocarbon as its cave counterpart. Expressed per unit body weight, these values convert to 1.93 mg g^{-1} vs. 0.63 mg g^{-1} (lipid) and 0.18 mg g^{-1} vs. 0.07 mg g^{-1} (hydrocarbon) for the epigean and cave

Table 2.—Hydrocarbon composition (%) of *Lycosa howarthi* and *Lycosa* sp. cuticular lipids. Only components accounting for 0.1% or greater of the total HC fraction are listed. Values represent means of three replicate runs on each group. ECL = equivalent chain length; tr = trace (less than 0.1%);b = branched.

GLC peak no.	ECL	*Lycosa howarthi*	*Lycosa* sp.
18	18	.40	tr
19	19	1.07	.22
19b	19.2	—	.37
19b	19.6	.51	.54
20	20	3.13	.83
21	21	4 38	1.92
21b	21.6	2.98	1.45
22	22	3.17	1.37
22b	22.4	2.85	1.60
23	23	4.33	1.47
23b	23.3	—	.83
23b	23.7	—	.62
24	24	4.36	1.39
24b	24.3	1.72	.53
24b	24.7	—	.65
25	25	4.05	2.22
25b	25.3	—	.45
25b	25.7	.70	.56
26	26	2.84	1.26
26b	26.4	.40	—
26b	26.7	.54	1.57
27	27	3.29	4.01
27b	27.3	—	.49
27b	27.7	—	.95
28	28	4.24	1.99
28b	28.3	—	.15
28b	28.7	.47	1.75
29	29	6.24	6.21
29b	29.3	—	3.86
29b	29.7	—	3.15
30	30	5.06	.60
30b	30.4	—	.34
31	31	5.03	.77
31b	31.3	—	1.85
31b	31.6	—	1.86
32	32	3.67	.41
32b	32.3	—	2.18
32b	32.7	—	.50
33	33	3.43	.75
33b	33.3	—	3.00
33b	33.7	.54	9.71
34	34	2.33	tr
34b	34.3	—	9.48
34b	34.7	.82	.66
35	35	2.20	—
35b	35.5	1.18	5.14
36	36	1.41	—
36b	36.4	1.85	2.08
37b	37.3	1.93	2.81
37b	37.7	2.50	7.17
38b	38.4	4.70	.52
39b	39.5	3.80	5.71
40b	40.3	1.40	2.13
40b	40.8	1.01	—
41b	41.7	5.05	—
	n-alkanes	64.9%	23.5%
	branched alkanes	35.1%	76.5%

species, respectively. The ratio of hydrocarbon to lipid was virtually identical for both species. No attempt was made to quantify or further analyze any of the non-hydrocarbon cuticular constituents.

Gas chromatographic analysis of the hydrocarbon fraction of *Lycosa* sp. revealed 48 separable components versus 39 for *Lycosa howarthi* (Table 2). In both species the hydrocarbon molecules were saturated and ranged from approximately 18 to over 40 carbon atoms in length. A major difference was the predominance of straight-chain (*n*-alkane) molecules in the cave species and branched molecules in the epigean species. This difference resulted from the absence of branched components that correspond to the *n*-alkanes of the same carbon number (especially between C_{27} and C_{33}) in the cave species (Table 2). Long-chain branched molecules characterized the hydrocarbon fraction of both species. The hydrocarbon components were present in relatively equal amounts, particularly in the cave species where only one molecule accounted for more than 6% of the total hydrocarbon fraction. Although there was not enough material to permit mass spectroscopic confirmation of branching types, fractional equivalent chain lengths (0.3-0.4 and 0.6-0.7) suggest the presence of at least two homologous series of methyl branched components.

DISCUSSION

The habitats occupied by the two lycosid spiders examined in this study represent highly contrasting physical environments. The true deep cave zone, inhabited by *Lycosa howarthi*, features a relatively stable climate where temperatures are moderate (19 ±1°C) and evaporation absent or negligible. It is a rigorous environment in that it is perpetually dark and food-limited. Ecological studies including a food web analysis were reported in Howarth (1973) and Gagné and Howarth (1975). The epigean wolf spider (*Lycosa* sp.) occurs on barren to semi-vegetated flows of Kilauea Volcano (Howarth 1979). Although annual rainfall ranges between 1100 and 2000 mm, the surface appears extremely xeric due to rapid evaporation from the black lava, the poor water-holding capacity of lava, the rapid percolation of rain water into the porous rock, and the high drying power created by the almost constant wind. A 25° to 50°C difference between the daily maximum and minimum surface temperatures is not uncommon. It is not surprising that *Lycosa* sp. individuals hide in cracks and under large rocks during the day and only venture onto the surface at night.

The water loss rates (WLR) for the two spider species correspond to the environmental stresses imposed by their respective habitats. In the troglobitic spiders, which normally experience saturated atmospheres, WLR increased markedly with a reduction in relative humidity (i.e., increased saturation deficit), reaching a maximum of 11.14 mg g^{-1} h^{-1} at 19°C and 0% RH (Fig. 1). In contrast, WLR for the lava flow spiders, which are subjected to higher saturation deficits, were relatively independent of changes in relative humidity between 0 and 70% (Fig. 1). Higher transpiration rates were also found in the cave-dwelling cockroach *Blaberus craniifer* Burmeister (Herreid 1969) and the troglobitic collembolan *Tomocerus problematicus* (Vannier 1979) than in their epigean counterparts *Periplaneta americana* (L.) and *Tomocerus minor* (Lubbock), respectively.

Under the experimental conditions employed in this study (low temperature, post-absorptive animals), the total water loss rates for the spiders essentially represent their cuticular transpiration rates and, hence, permit discussion in terms of cuticular permeability. For this purpose it is convenient to use area-specific water loss rates corrected for

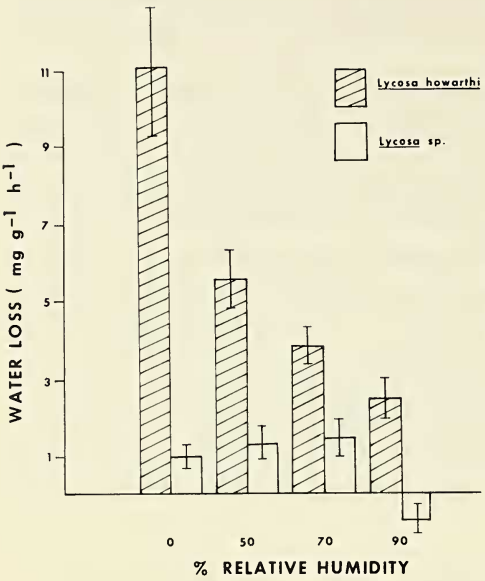

Fig. 1.–Mean water loss (gain) rates of the troglobitic spider, *Lycosa howarthi*, and the lava flow spider, *Lycosa* sp. at 19°C and various relative humidities. Sample size at each test humidity was seven except for 90% (n = 6). Vertical bars represent ± 1 SE.

saturation deficit (i.e., $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$) (Edney 1977). At 19°C and 0% RH, permeability values for the troglobitic spider were 33.4 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ versus 3.1 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$ for the epigean lycosid. Although the cuticular permeability of the cave species is 10 times higher than the lava flow species, it is only slightly greater than the permeability for the mesic-adapted spider, *Lycosa amentata* (Clerck) (28.3 $\mu g\ cm^{-2}\ h^{-1}\ mmHg^{-1}$) (Davies and Edney 1952), and is significantly lower than values given for several species of Australian cave-dwelling crickets (Campbell 1980). The cuticular permeability of the lava flow spider is typical of the most xeric-adapted insects and arachnids (Edney 1977) and no doubt is an important factor in their ability to inhabit the harsher surface lava environment.

The difference in cuticular permeability between *Lycosa howarthi* and *Lycosa* sp. is likely due in part to differences in their surface lipid and hydrocarbon densities. The epigean species possessed greater amounts of both cuticular lipid and hydrocarbon on an individual spider and weight-specific basis (Table 1). The hydrocarbon fraction, which is effective in waterproofing the surface of many plants and animals (Hadley 1980), was nearly three times more abundant in the epigean spiders (weight-specific) and nearly twice as abundant when expressed per unit surface area (.007 vs .004 mg cm^{-2}). The hydrocarbon density for *Lycosa* sp. is virtually identical to values for xeric-adapted black widow spiders, *Latrodectus hesperus* Chamberlin and Ivie (Hadley 1978). The correlation between cuticular permeability and the composition of the hydrocarbon fractions of the two Hawaii Island *Lycosa* species is not as clearly defined. Hydrocarbons of both species were composed of numerous, saturated components, and the range of chain lengths represented were essentially the same (Table 2). A notable difference between the two spider species was the abundance of branched alkanes in the epigean spiders and the predominance of *n*-alkanes in the cave spiders. A higher percentage of long-chain, branched molecules characterized the hydrocarbon fraction of the desert-adapted scorpion, *Hadrurus arizonensis*, Ewing and another scorpion species, *Centruroides sculpturatus* Ewing, active during hot summer months when compared with a mesic-adapted scorpion, *Uroctonus apacheanus* Gertsch and Soleglad, and *C. sculpturatus*, collected during winter,

respectively (Toolson and Hadley 1977, 1979). In the present study, however, the higher frequency of branched molecules in the epigean species is evident only in the mid-range of its hydrocarbon spectrum (Table 2). Compositional differences in the hydrocarbon fraction of the two spider species do indicate either major differences in their diet and/or genetic isolation over a sufficiently long period to permit changes in the enzymes responsible for synthesis of the branching types present.

The metabolic rate data for the two spider species are very preliminary and must be viewed with caution even though observed rates coincide with published values for arachnids and specifically spiders under comparable test conditions. The mean (±1 SE) oxygen consumption for the lava flow spider (173.72 ±6.22 μl O_2 g^{-1} h^{-1}) over the 12-h period from 1500 to 0300 h (19°C) is significantly higher ($P < 0.01$) than the mean recorded for the cave species (115.71 ± .89 μl O_2 g^{-1} h^{-1}). Both values fall within the range of metabolic rates (21-356 μl O_2 g^{-1} h^{-1}) reported for arachnids at 20°C by Anderson (1970) as well as for lycosid spiders (92.8-452.2 μl O_2 g^{-1} h^{-1}) at 20°C (calculated from data presented by Humphreys 1977). The metabolic rates of arachnids are typically lower than those of poikilotherms of comparable size and, in the case of the cave species, falls at the lower end of the metabolic spectrum for lycosid spiders. This finding is consistent with the hypothesis (Anderson 1970) that a comparatively low metabolic rate for arachnids (spiders) is an adaptation to the potential problem of being faced with an inconsistent food supply, a situation likely to occur in the deep cave zone of the Hawaiian lava tubes (Howarth 1973). It is also consistent with the "sit and wait" predatory behavior exhibited by *Lycosa howarthi* in the cave environment (Howarth, unpub.).

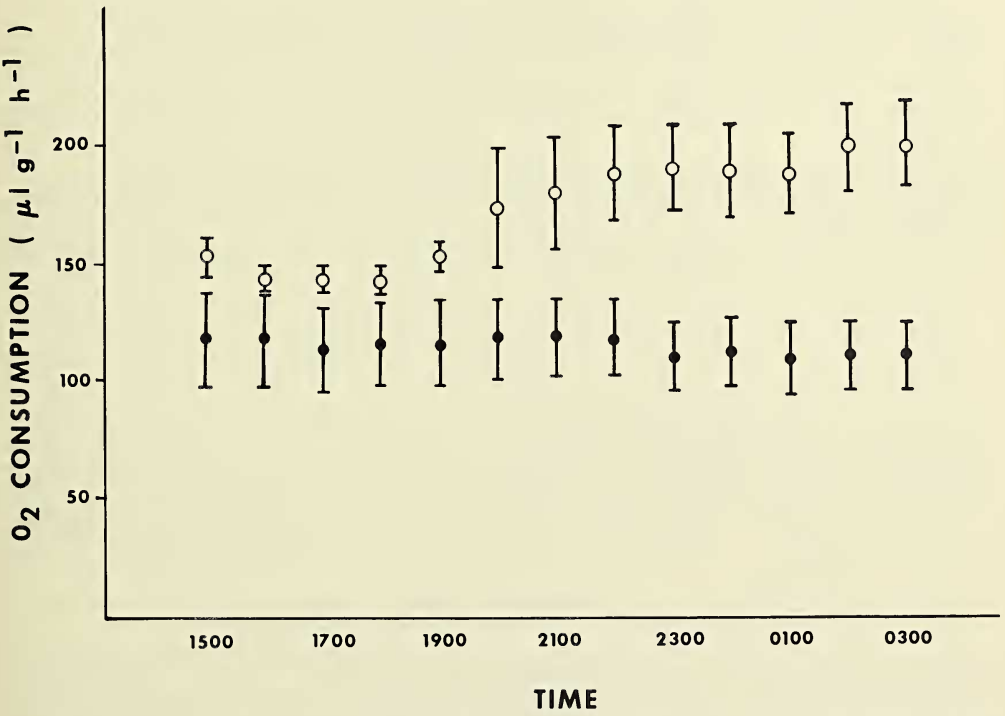

Fig. 2.–Mean oxygen consumption rates of the troglobitic spider, *Lycosa howarthi* (closed circles) and the lava flow spider, *Lycosa* sp., (open circles) at 19°C between 1500 h (3 PM) and 0300 h (3AM). Vertical bars represent ± 1 SE. Minimum sample size for each species was four.

The preliminary metabolic data (Fig. 2) suggest an increased oxygen consumption rate for the epigean species at approximately the time it would be active in nature, and the apparent lack of any cyclic rhythm in the cave species. A cyclic rhythm in oxygen consumption could be expected in the epigean species which routinely leaves the protection of daytime cover and moves to the surface at night to feed. The apparent absence of any metabolic rhythm in the cave species is also not surprising since it remains in a stable environment with no obvious environmental cue to initiate feeding or locomotor behavior. Additional tests using larger sample sizes, a variety of ambient conditions, and spiders segregated as to sex and age are necessary to confirm these preliminary observations.

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